Metabolic Activation/Deactivation Reactions during Perinatal Development

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The role of metabolic activation/deactivation reactions during development is evaluated in relation to developmental pharmacology and toxicology. Enzyme systems evaluated include the mixed-function oxidases (aryl hydrocarbon hydroxylase and oxidative demethylation), epoxide hydration and conjugation (glutathione conjugation, sulfation, and glucuronidation). Placental transfer and milk secretion of chemicals are discussed in relation to maternal, placental, and fetal metabolism. Normal patterns of enzyme development can be modified in two ways: (1) enzyme induction and (2) enzyme imprinting. Postnatal induction of the mixed-function oxidases and glucuronyl-transferase following treatment of pregnant rats with TCDD is shown to be caused primarily by newborn exposure to TCDD in milk. Structure-activity relationship are defined for the perinatal induction of hepatic enzymes by the pure PCBs. PCBs are divided into two classes: P-450 inducers and P-448 inducers. Imprinting or programming of hepatic metabolism is a function of the sexual differentiation of enzyme activity; male and female activities are similar in prepubertal animals, whereas pronounced sex differences are evident in adults. Treatment of newborn rats (days 2–6) with diethylstilbestrol or testosterone resulted in a feminization (decrease) of mixed-function oxidation and glucuronidation in adult males. No changes were seen in immature males or females or adult females. This effect appears to be irreversible and is under pituitary-hypothalamic-gonadal control. In addition to the feminization of enzyme activity, neonatal exposure to hormonally active chemicals also feminizes the hepatic response to cadmium in resultant adult animals.

The role of metabolism in xenobiotic pharmacokinetics and toxicity has received considerable attention over the last two decades. The concept of metabolic activation of chemicals to reactive intermediates is critical in attempts to characterize the sequence of molecular events eventually leading to environmental carcinogenesis, mutagenesis and teratogenesis.

A theoretical scheme whereby chemicals might initiate carcinogenesis could begin by exposure to an inactive precursor. The precursor could be converted to a reactive intermediate, presumably by the hepatic or extrahepatic monooxygenase system catalyzed by cytochrome P-448 or P-450. Reactive intermediates are thought to be electrophilic (often epoxides), and they can bind covalently to nucleophilic nitrogen, oxygen, or sulfur atoms or cellular macromolecules (1). Damaged macromolecules can result from this interaction resulting in the initiation of carcinogenesis. Conversely, an inactive precur-

sor may be enzymatically deactivated by a wide variety of enzyme systems including the monooxygenase system. Moreover, a reactive intermediate may be deactivated prior to the initiation of carcinogenesis (Fig. 1). These considerations are applicable to both adult and developing systems.

Factors other than metabolic deactivation may play a role in preventing carcinogenesis and other forms of toxicity. For example, DNA repair processes may prevent the initiation of toxicity following co-

Active Parent Compound ⟶ Inactive Metabolites

Inactive Parent Compound ⟶ Inactive Metabolites
↓
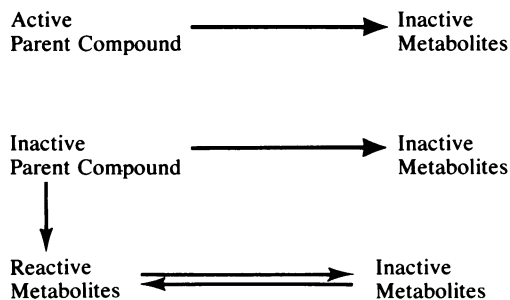Reactive Metabolites ⇄ Inactive Metabolites

FIGURE 1. Diagrammatic pathways for metabolic activation/deactivation of foreign chemicals.

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valent binding. If, these repair processes are present in fetal tissues they could modify the expression of transplacental toxicity. Immunological defenses are also operative in perinatal tissues (2) and might function to stop the progression of toxicity at various developmental stages.

Factors in Activation/Deactivation Reactions

The role of activation/deactivation reactions in drug disposition and transplacental toxicity during development is not well understood and is complicated by a large number of variables not encountered in mature systems (3). Some of these factors are outlined in the following paragraphs.

Differing Developmental Patterns of Specific Reactions

The balance between activation and deactivation reactions for specific substrates fluctuates dramatically in an age-dependent manner. It is difficult to predict whether a specific enzyme system will function as an activation or deactivation system. Table 1 lists the potential dual role of several of the drug-metabolizing enzymes that have been postulated in experimental animals, according to factors such as age, nature of the substrate, and site of metabolism. One of the greatest problems in elucidating the role of metabolism in toxicity is characterization of the mechanisms of toxicity including the identity of the active metabolite. There is much controversy on this subject. For example, it is not clear whether the potent transplacental carcinogen, diethylstilbestrol initiates its toxic actions through an active metabolite or the estrogen action of the parent compound (4).

Relative Importance of Maternal and Fetal Metabolism

An important factor in transplacental toxicity evaluation involves the capacity of fetal tissues to metabolize xenobiotics. If bioactivation occurs only in the maternal compartment, the physical properties and stability of the reactive metabolite determine access to the fetal compartment. Clearance of both active and inactive metabolites from the fetus often occurs much slower than in adults.

Role of Metabolism in Placental Transfer of Chemicals

There are a number of factors that modify placental transfer rates of chemicals, including polarity (5). Metabolic reactions such as hydroxylation and conjugation can occur in maternal or placental compartments and generally decrease lipophilicity of xenobiotics and therefore retard placental transfer. In contrast, reactions such as deconjugation increase lipophilicity and therefore increase transfer rates from the maternal to fetal compartment (6).

Relative Importance of Hepatic and Extrahepatic Metabolism during Development

During recent years the role of extrahepatic drug metabolism in organ specific toxicity and pharmacokinetics has become apparent (7). One of the clearest examples involves colon carcinogenesis induced by dimethylhydrazine as a function of intestinal metabolism and associated enterohepatic circulation of this chemical (8). It therefore becomes important to ascertain the balance between activation and deactivation reactions of specific chemicals in specific tissues at specific ages to understand the role of drug metabolism in developmental toxicity.

Chemical and Disease-Mediated Alterations in Developmental Patterns of Drug-Metabolizing Enzymes

These enzyme systems are, in many cases, extremely sensitive to inductive or repressive actions of both endogenous and exogenous chemicals (7) and sensitivity to these actions appears to change with development (9, 10). Disease conditions during pregnancy might also significantly alter ontogeny of metabolic competence. Therefore, pre-existing disease conditions or co-exposure to environmental agents may modify toxic responses.

Sex Differences in Developmental Patterns

Sex differences exist in hepatic metabolism of many species and usually become evident only after sexual maturation (11). For example, rat mixed-function oxidase activity in liver is usually much greater in adult males than females, although differences in these enzyme systems are not apparent in

Table 1. Dual role of drug-metabolizing enzymes.

Enzyme reaction	Activation	Deactivation
Epoxidation	+	+
Hydroxylation	+	+
Glucuronidation	+	+
Epoxide hydration	+	+
Sulfation	+	+

immature animals (*12*). Sex differentiation of metabolic activation/deactivation reactions may lead to sex as well as age-dependent differences in toxicity.

Extrapolation of Animal Data to the Human Condition

Extrapolation is complicated by many factors. One difficulty arises from the qualitative as well as quantitative differences that exist in the ontogeny of drug metabolizing enzymes of different species. For example, human fetuses have significant levels of mixed-function oxidase activity, whereas the onset of activity of this system in experimental animals does not occur until parturition (*10, 13–15*). However, mixed-function oxidase activity in human fetuses may reflect transplacental exposure to polycyclic hydrocarbons which are capable of inducing enzyme activity in fetuses of experimental animals (*10, 16*). Moreover, the perinatal response of drug-metabolizing enzymes to inducing agents differs according to strain as well as species (*15*).

Microsomal Ontogeny of Monoxygenases

Aryl Hydrocarbon Hydroxylase

Aryl hydrocarbon hydroxylase (AHH) is part of a cytochrome P-450-dependent enzyme system termed collectively the mixed-function oxidase or monooxygenase system. This microsomal enzyme complex functions to metabolize a large number of xenobiotics having widely different structural components and spectrum of toxic actions. AHH is often used as an indicator of the capacity of biological systems to form reactive electrophilic metabolites which are frequently in the form of arene oxide intermediates (*17*) produced in hydroxylation reactions. However, AHH assays often are a measure of total hydroxylated or polar metabolites and are not necessarily indicative of metabolic activation. For example, polycyclic hydrocarbons such as benzo[a]pyrene generate large numbers of microsomal oxidative metabolites possessing widely different capacities for binding to cellular macromolecules in *in vitro* systems (*18–20*). Information on the rates of formation of specific active metabolites capable of eliciting biochemical lesions is needed to achieve a valid toxicokinetic evaluation. However, such a definitive evaluation is not feasible for each toxicant. Therefore, determinations of AHH activity by using benzpyrene, biphenyl, or other suitable substrate may provide approximate data on the capacity of some biological systems to activate some substrates (those activated by the microsomal monooxygenase system).

Characterization of the role of metabolic activation/deactivation in transplacental toxicity is extemely difficult in developing and differentiating systems. Nevertheless, information on the ontogeny of AHH is available and a schematic representation of AHH ontogeny in rat liver is presented in Figure 2. This scheme was derived as a composite of several studies (*10, 16, 21*) and unpublished observations. Enzyme activity is first detectable just prior to birth and increases rapidly for the first two weeks of life, corresponding to the ontogeny of cytochrome P-450. AHH activity then plateaus until puberty, when a sexual differentiation of enzyme activity is expressed: male liver enzyme activities become approximately 6-fold higher in males than females (*21*). Sexual differentiation of the monooxygenase system is pituitary-dependent and appears to be programmed by neonatal exposure to testicular androgens during a critical period of early development (*12, 22*). Biphenyl hydroxylation also exhibits a sex difference in adult rats (*23*), whereas hydroxylations of aniline and zoxazolamine do not exhibit sex differences (*11*). The neonatal development of AHH appears to be similar in rabbits (*24*), mice (*25*), and guinea pigs (*26*).

Unlike the rat, human fetuses contain significant levels of AHH activity in early gestation (*13, 27*). Moreover, AHH activity has been detected in extrahepatic organs of human fetuses: adrenal, vagina, uterus, testis, ovary, intestine, kidney, lung, and placenta (*28, 29*).
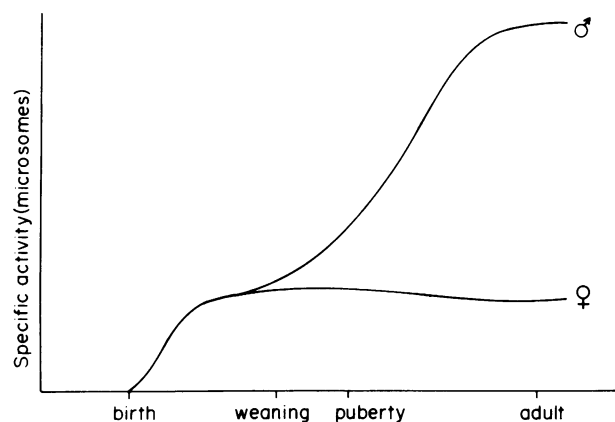

FIGURE 2. Schematic representation of aryl hydrocarbon hydroxylase ontogeny in rat liver. This scheme was derived as a composite of many studies (*10, 16, 21*, and unpublished observations).

N-Hydroxylation

N-Hydroxylation is potentially an important metabolic pathway in the evaluation of transplacental toxicity as a function of subsequent formation of reactive electrophilic esters of N-hydroxy metabolites (30). Fetal and placental tissues of humans and monkeys are capable of ring- and N-hydroxylation of the potent carcinogen 2-acetylaminofluorene (31). N-Oxidation first occurs in rabbits just prior to birth, followed by a gradual increase in activity until adult levels are attained (32).

N-Demethylation

A schematic representation of oxidative N-demethylation ontogeny in rat liver microsomes is presented in Figure 3, which is a composite derived from the works of several laboratories (12, 22, 33, 34). Enzyme activity is first detected just prior to birth followed by an increase up to two weeks. Demethylation rates then plateau until puberty when, as with AHH, a pronounced sexual differentiation
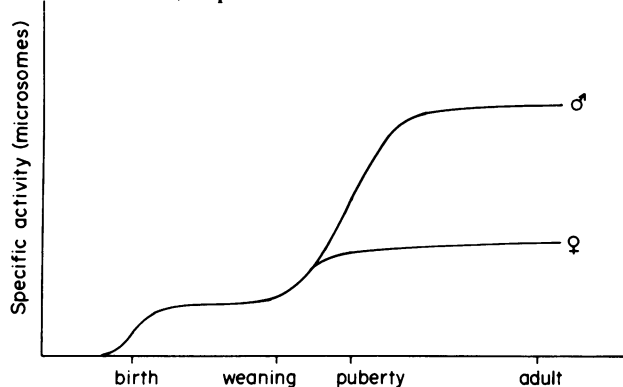
FIGURE 3. Schematic representation of oxidative N-demethylation ontogeny in rat liver. This scheme was derived as a composite of several studies (12, 22, 33, 34).
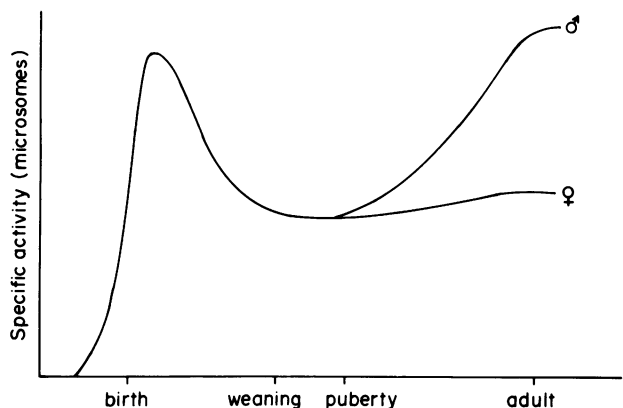
FIGURE 4. Schematic representation of glucuronidation rates of one group of substrates by UDP glucuronyltransferase. This scheme was derived as a composite of several studies (10, 33, 38, 39).

of activity occurs. Oxidative demethylation is easily detected in human fetuses at levels approximately 30% those of human adults (13, 14, 27).

Ontogeny of Conjugation Reactions

On simplification, and depending on chemical substrate the drug biotransformation process may be separated into two parts: first an oxidative reaction, such as hydroxylation, results in the formation of a free hydroxyl group; this is then rapidly conjugated with glucuronic acid, sulfate, or another conjugate (35) as indicated in Eqs. (1) and (2). This
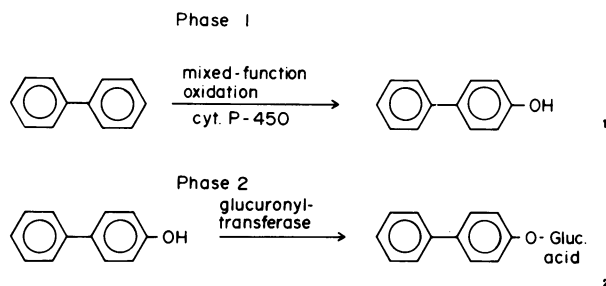
series of reactions renders the molecule more polar and generally more excretable and less toxic. However, conjugation reactions may also function in the formation of reactive electrophilic intermediates (30, 36, 37) (Table 1).

UDP Glucuronyltransferase (UDPGT)

A schematic composite of UDPGT ontogeny for one group of substrates in rat liver is presented in Figure 4 (10, 33, 38, 39). The onset of UDPGT activity occurs 5 days prior to birth followed by a dramatic increase in activity such that glucuronidation rates at birth exceed adult levels. Glucuronidation rates for a second group of substrates do not exhibit this developmental peak (10, 38, 39), providing one criterion used to ascertain functional heterogeneity of UDPGT. Purification procedures have recently separated two forms of UDPGT (40, 41). UDPGT, like the microsomal monooxygenase system, exhibits sexual differentiation.

The elucidation of the factors that regulate onset of glucuronidation has received considerable attention. Experiments which chick embryo liver cultures have demonstrated that UDPGT activity increases markedly in culture and actually exceeds that found normally in adult liver (42). The conclusion is that developmental onset of glucuronidation appears to be regulated by UDPGT synthesis. Also, it has been reported that the onset of UDPGT in chick embryo liver culture is under pituitary control

through production of active glucucorticoids (43, 44). Moreover, it appears that the differential ages for the onset of UDPGT activity in different tissues is probably related to tissue-specific activation of UDPGT synthesis (45). The characteristic developmental peak for nonsteroid UDPGT is not seen in rabbit liver and is also not seen in kidney and lung (45). Extrahepatic tissues in fetuses of experimental animals do not contain significant UDPGT although some tissues such as kidney have high levels in adults (45). UDPGT activities are low or nondetectable in human fetuses (46).

Sulfotransferase

Sulfotransferase is a family of cytosolic enzymes which catalyze the transfer of active sulfate from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to steroids and xenobiotics (35). Sulfation is generally considered a detoxication reaction, but the sulfate ester of the N-hydroxy metabolite of AAF is a reactive electrophile (30, 37). Moreover, sulfated steroids are important to hormone action (47). Although data on the ontogeny of sulfotransferase is limited, ontogeny and partial purification studies have demonstrated enzyme multiplicity (48). Sexual differentiation of sulfotransferase is substrate-dependent for a variety of glucocorticoids and steroids (48). No information was found on the perinatal development of sulfation of xenobiotics in either humans or experimental animals.

N-Acetyltransferase

N-Acetylation is one of the principal mechanisms for the metabolism of foreign arylamines and arylhydrazides in most mammalian species (35). This process involves the transfer of the acetyl moiety of acetyl coenzyme A (acetyl CoA) to arylamines by N-acetyltransferase. N-Acetyltransferase is located in the soluble portion of mammalian liver and has been considered to be a detoxication enzyme. Perinatal development studies in rats and rabbits revealed that fetal and neonatal animals are capable of N-acetylation (49). Rat liver developmental studies demonstrated two peaks of activity with the first peak occurring in the late fetus followed by a second peak three days after birth. Rabbit fetal and neonatal enzyme activity increased to adult levels by the second week after birth in liver and gut; however, lung showed a different developmental pattern. The enzyme has been partially purified from livers of developing rabbits, and it was found that fetal sulfamethazine N-acetylation rates were considerably less than those observed for adults, and activity reached adult

levels at about one month of age (50). Kinetic determinations of rabbit liver N-acetyltransferase provided evidence to correlate developmental variations with a "fetal" enzyme form.

Ontogeny of Epoxide-Metabolizing Enzymes

As discussed earlier in this paper, many aromatic and olefinic xenobiotics are metabolized to epoxides (arene or alkene oxides) by the microsomal monooxygenase system. These reactive intermediates can bind covalently to DNA, RNA and proteins to initiate a toxic response; carcinogenesis, mutagenesis, teratogenesis or organ specific toxicity. Epoxides can be further metabolized by epoxide hydratase to form a diol or by a complex or cytosolic enzymes known collectively as glutathione S-transferase. Both epoxide metabolizing enzymes function to deactivate epoxides (51). However, epoxide diols of benzpyrene are reactive electrophiles (52), suggesting that epoxide hydratase may play an indirect role in the formation of biologically reactive metabolites. Developmental studies in mice, guinea pigs, and rabbits have demonstrated that ontogeny of epoxide hydratase and glutathione S-transferase is organ-specific; liver, lung, intestine and kidney were investigated (53, 54). Enzyme activity of both epoxide metabolizing systems is present in fetal tissues, in some cases equal to adult levels. In general the ontogeny of epoxide hydratase is similar to and is related to the development of AHH and other microsomal monooxygenase components (54, 55).

Modifications of Ontogeny

The microsomal monooxygenase system and many of the conjugative enzymes, most notably UDPGT, can be induced or repressed by a wide variety of chemicals including polycyclic hydrocarbons, halogenated aromatics, barbituites, hormones, and many others. Considerable information is also available on the perinatal effects of these chemicals on metabolic activation/deactivation reactions following transplacental and/or lactational exposures. Information in the present paper will emphasize alterations in the development of AHH and UDPGT following perinatal treatment of experimental animals with halogenated aromatics. In a second section, the capacity for neonatal hormones to irreversibly alter the metabolic competence of subsequent adult rats, will be evaluated.
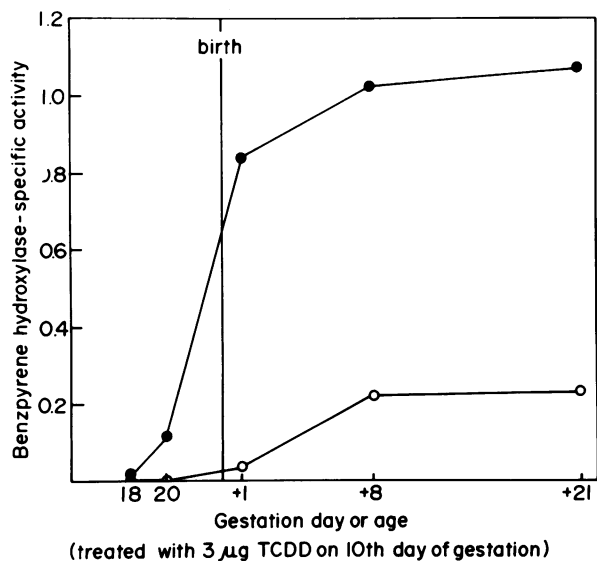
FIGURE 5. Effects of treatment of pregnant rats with 3 μg TCDD/kg on ontogeny of hepatic aryl hydrocarbon hydroxylase. Each point derived as an average of at least four separate litters. Assay methods presented elsewhere (21).

Perinatal Induction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been detected as a contaminant of some organochlorines, most notably the herbicide 2,4,5-T. This chemical is extremely toxic and is also a potent inducer of specific microsomal mixed-function oxidases and UDP glucuronyltransferase in several tissues of adult rats (56). The effects of administration of TCDD to pregnant rats on the ontogeny of some hepatic drug-metabolizing enzymes was investigated (10). Results reveal that AHH was markedly induced in fetal rat liver (Fig. 5). Moreover, the onset of enzyme activity was detected in fetal livers four days prior to birth in the treated group compared to two days prior to birth in the control group (10).

Later studies have investigated the transplacental induction of carcinogen hydroxylating systems by TCDD (16) AHH was induced nearly 100-fold in fetal rats but only minimal increases were evident in placentas. Rates of ring and N-hydroxylation of N-2-fluorenylacetamide were also markedly induced. Increased formation of benzyprene diols in both the fetal and maternal compartments was associated with the increased AHH activity in fetal rat livers. Therefore, it appears that the lack of response of fetal enzymes to many inducing agents is not due to the lack of an appropriate genetic mechanism in fetal liver but is likely a function of pharmacokinetic factors such as maternal metab-

olism, placental transfer and/or xenobiotic-receptor interactions. In contrast to AHH, fetal hepatic steroid and nonsteroid UDPGT are unaffected by TCDD treatment of the pregnant animal (Figs. 6 and 7). Increased activity of AHH and nonsteroid UDPGT is evident postnatally, whereas steroid UDPGT was unchanged in the same animals. Differences in susceptibility to TCDD inductive actions, tissue distribution, and enzyme ontogeny have been used as criteria for the contention that steroid and nonsteroid UDPGT are at least two separate enzyme systems (10, 38, 39, 57). UDPGT activity in extrahepatic tissues is also induced in offspring of pregnant rats administered TCDD (21). The neonates have elevated levels of UDPGT in liver, kidney, lung, and intestine, but like adult rats, testicular tissue is nonresponsive. Increased levels of enzyme activity are most pronounced in the kidney.

Since nonsteroid UDPGT is induced postnatally but not prenatally following treatment of pregnant rats with TCDD, pups were crossed-fostered immediately after birth to determine whether the postnatal stimulation of UDPGT is related to newborn exposure to TCDD in milk or to the postnatal activation of an inducing mechanism (10). Results revealed that the major factor is newborn exposure to TCDD in milk although UDPGT in the postnatal liver does appear to be more responsive to TCDD than the prenatal liver. Tissue distribution experiments with ¹⁴C-radiolabel demonstrate the TCDD
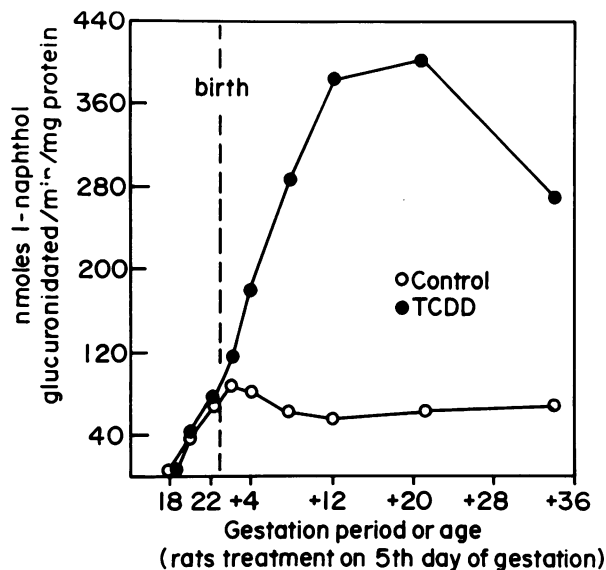

FIGURE 6. Effects of treatment of pregnant rats with 3 μg TCDD/kg on the ontogeny of hepatic 1-naphthol glucuronyltransferase activity. Each value derived from an average of at least four separate litters. Assay methods presented elsewhere (38).
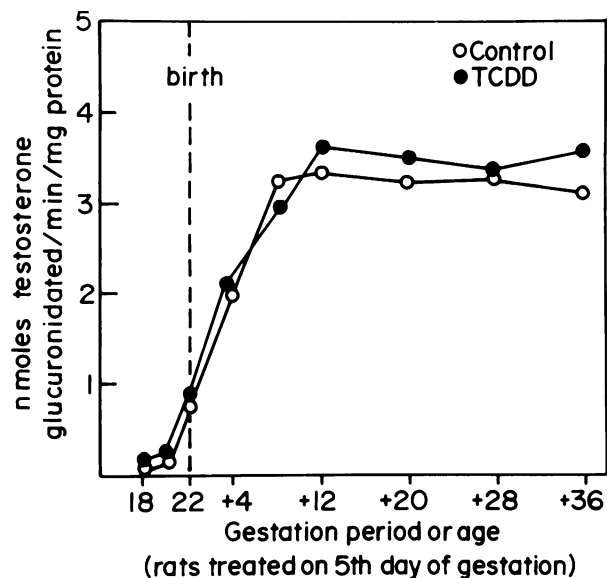
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FIGURE 7. Effects of treatment of pregnant rats with 3 μg TCDD/kg on the ontogeny of hepatic testosterone glucuronyltransferase activity. Each value derived from at least four separate litters. Assay methods presented elsewhere (38).

does not readily cross the placenta but is apparently secreted in high concentrations in milk. Postnatal induction of microsomal enzymes by newborn exposure to organohalogens in milk is not a unique property of TCDD. Neonatal mice which had received kepone and mirex exhibit dramatic elevations in oxidative O- and N-demethylation pathways (58). The PBBs are also effective transplacental inducers (induction characteristics are mixed with some phenobarbital type induction and some 3-methylcholanthrene type induction) of the hepatic monooxygenase system (59) but are also secreted in high concentrations in milk resulting in increased enzyme activity in pups receiving PBBs only via lactation (59, 60).

The polychlorinated biphenyls (PCBs) are produced commercially as a mixture of many congeners and are generally considered to be biologically—and ecologically—persistent industrial pollutants. Most of the toxicology studies have been derived by using these mixtures. Such studies provide important and necessary toxicological data but reveal little about structure–activity relationships of individual congeners differing in the number and position of chlorine substitutions. PCBs have teratogenic potential, as evidenced by skin, liver, and growth disorders in offspring of pregnant women exposed to PCBs in Yusho, Japan (61).

Pharmacokinetic studies reveal that the presence of two unsubstituted carbon atoms on the biphenyl ring markedly enhance the metabolic rate constant

of PCBs in adult rats (62). Recent studies in our laboratory have characterized some of the pharmacologic properties of pure PCB congeners in developing rats (63).

Analysis of structure–activity relationships governing the perinatal pharmacology of the PCBs and PBBs provided guidance for selecting representative congeners for evaluating the effects of PCBs on ontogeny of hepatic enzymes. Accordingly, pregnant rats were treated with 3,4-3′4′-tetrachlorobiphenyl (4-CB) or 2,4,5-2′,4′,5′-hexachlorobiphenyl (6-CB), and developmental patterns of several hepatic enzymes were investigated: ethoxyresorufin deethylase (cytochrome P-448-dependent), aminopyrine demethylase (cytochrome P-450-dependent), 16α-hydroxylase (cytochrome P-450-dependent), 5α-reductase, and UDPGT. Data revealed (manuscript in preparation) several interesting findings. 4-CB induces cytochrome P-448-dependent enzymes and 6-CB induces P-450-dependent pathways in livers of offspring exposed to these congeners transplacentally and during lactation. Thus, the perinatal and adult livers respond to PCBs in much the same way (64–66). Our studies demonstrate that in developing rats, like adults, individual congeners are P-450 or P-448 inducers and that neonates may be more sensitive to PCB-mediated increases in enzyme activities than adults. However, conclusions regarding age-dependent sensitivities to induction of hepatic enzymes must await quantitation of parent compound and/or toxic metabolites of individual congeners in various tissues during perinatal development. UDPGT, which is not P-450 or P-448 dependent is induced by both 4-CB and 6-CB in neonatal rat livers. In contrast to other hepatic enzymes, activities of the hepatic steroid-metabolizing enzyme, 5α-reductase, are markedly repressed by 6-CB but not 4-CB in neonatal rats (67). The mechanism of repression and the physiological significance of this finding have not been characterized.

Imprinting of Enzyme Activity

Enzyme induction, as discussed in an earlier part of this report, probably results from direct action of the chemical or chemical-receptor with genetic material resulting in an immediate modulation of enzyme levels as a function of increased enzyme synthesis. A second form of enzyme modulation which is initiated neonatally has been termed "imprinting" or programming of enzyme activity. Imprinting is manifested through neonatal exposure to hormones or hormonally active agents and results in an irreversible determination of behavioral characteristics and/or enzyme levels in the sub-

Table 2. Effects of neonatal diethylstilbestrol or testosterone proprionate (TP) on the sexual differentiation of rat hepatic enzymes.[a]

	Adult male/female specific activity ratios			
Enzyme	Control	Neonatal DES	Neonatal TP	Hypophysectomized
Ethylmorphine demethylase	4.2	1.3	1.6	1.0
Cytochrome P-450	1.1	1.0	1.0	1.0
UDPGT	1.9	1.2	1.4	1.0

[a] Data summarized from the literature (22, 70, 71).

Table 3. Effects of neonatal DES or TP on the response of hepatic enzymes to cadmium in adult rats.[a]

	Repression by Cd (2 mg/kg, IP)		
Enzyme	Control	Neonatal DES	Neonatal TP
Ethylmorphine demethylase, male	+	−	−
Ethylmorphine demethylase, female	−	−	−
Cytochrome P-450, male	+	−	−
Cytochrome P-450, female	−	−	−

[a] Data summarized from the literature (22, 70).

sequent adult animal (68, 69). These imprinted endpoints are, however, not evident in the prepubertal animal, suggesting a latency of gene expression resulting from hormone action during early development. The developmental patterns for many enzymes exhibit no sex differences in prepubertal animals. However, a postpubertal sexual differentiation of metabolic competence often occurs with the activities of one sex being markedly different than the other. Sexual differentiation of enzyme activity appears to be a function of neonatal estrogen or androgen exposure. UDPGT and many oxidative pathways exhibit higher activities in adult males than females (Figs. 2 and 4), as well as differences in kinetic constants (12). Following administration of DES or testosterone to neonatal rats the postpubertal sexual differentiation of the microsomal monooxygenase system is abolished (22, 70). This feminization of enzyme activity in male rats is accompanied by a feminization of the K_m value and turnover number for ethylmorphine demethylation suggesting that neonatal exposure to hormonally-active chemicals programs for both qualitative and quantitative alterations of the ontogeny of the microsomal monoxygenase system. Similar feminization effects were observed for UDPGT development following administration of DES or testosterone to neonatal male rats; ontogeny of female UDPGT is unaffected (71). Hypophysectomy of adult rats also abolishes the sex differences in the microsomal monooxygenase and UDPGT systems (Table 2) providing evidence for the contention that

neonatal hormone exposure programs the pituitary-hypothalamix axis, during a critical period of early development, for the sexual differentiation of many hepatic enzymes (12, 68-71). Studies on reproductive tract development and hormone levels in animals exposed neonatally to hormonally active chemicals suggests that altered gonadal development can also modify hepatic enzyme ontogeny (71).

The neonatal feminization of hepatic development also results in a feminized response of the adult liver to cadmium (22, 70). Cadmium administered to adult male rats represses the monooxygenase system whereas female enzymes are unaffected by cadmium (70). However, following neonatal TP or DES, resultant adult male and female rats respond in a similar manner (Table 3) (22, 70). These changes in toxic response are apparently irreversible and are manifested long after the affector (DES) is metabolized and excreted. Further studies are attempting to characterize the toxicological significance of alterations in the normal programing of enzyme activity emphasizing the potential role in developmental toxicity testing of environmental agents.

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